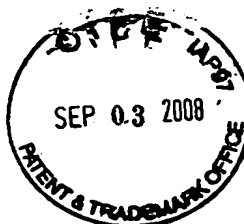


VERIFICATION OF TRANSLATION

I, Joichi OKAMURA, residing at 2-11-7-502, Misato, Misato-shi, Saitama 341-0024 Japan, hereby certify that to the best of my knowledge and belief, the attached document is a true translation into English made by me of Japanese Patent Application No. 2002-301433 filed on October 16, 2002.

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[List of Submission]

[Name of Submission] Specification 1

[Name of Submission] Abstract 1

[Proof] Need

[Document name] Specification

[Title of the invention] PLASMA PRODUCT OR SERUM PRODUCT AND  
METHOD FOR PRODUCING THE SAME

[Claims]

[Claim 1] A method for producing a human or animal plasma product or serum product comprising:

(a) a step of reducing leukocytes in the plasma originating from a human or animal, and

(b) a step of filtering using a virus removal membrane after the step (a).

[Claim 2] The method according to claim 1, wherein the virus removal membrane used in step (b) has an average pore diameter of 100 nm or less.

[Claim 3] The method according to claim 1 or 2, wherein the step (a) is a leukocyte-reducing step using a leukocyte removal filter.

[Claim 4] The method according to any one of claims 1 to 3, wherein the steps (a) and (b) are carried out under the condition of a temperature of 25-40°C.

[Claim 5] The method according to any one of claims 1 to 4, wherein the steps (a) and (b) are carried out under the condition of a pressure of 98 kPa or less.

[Claim 6] The method according to any one of claims 1 to 5, wherein the amounts of blood passing through in the steps (a) and (b) are 100-500 ml, respectively.

[Claim 7] The method according to any one of claims 1 to 6, wherein the treatment time for the step (b) is 40 minutes or less.

[Claim 8] The method according to any one of claims 1 to

7, wherein the virus removal membrane used in the step (b) has an average pore diameter of 75 nm or less.

[Claim 9] The method according to any one of claims 1 to 8, wherein the virus removal membrane used in the step (b) is a combination of a virus removal membrane having an average pore diameter of 75 nm and another virus removal membrane having an average pore diameter of 35 nm subsequent to the former membrane.

[Claim 10] A human or animal plasma product or a serum product produced by a method comprising the following steps (a) and (b):

(a) a step of reducing leukocytes, and

(b) a step of filtering using a virus removal membrane after the step (a).

[Detailed Description of the Invention]

[0001]

[Technical field where the invention belongs]

The present invention relates to a human or animal plasma product or serum product which is highly safe, and to a method for producing the same.

[0002]

[Background art]

Plasma or blood serum of humans or animals has a potential risk of being contaminated with viruses. Therefore, the possibility of being infected with high risk viruses such as the AIDS virus and various hepatitis viruses, in the case of humans, cannot be denied, if a blood product manufactured using plasma or serum as a raw material is used.

Methods for preventing viral infection involved in the use of these blood products have been proposed heretofore. For example, a chemical deactivating method using a surfactant or methylene blue has been known as a method for deactivating viruses in blood products. However, any of these methods has problems such as denaturation of proteins, requirements for complicated procedures for removing used chemical substances, and remaining of chemical substances in the finished products.

[0003]

A method of removing viruses using a membrane, on the other hand, is excellent as compared with the other methods, because this method does not cause proteins to become denatured, is free from a substantial decrease in the activity, and can increase safety of the products against viruses. For example, patent documents 1 and 2 disclose methods for securing high safety against the hepatitis C virus or AIDS virus by treating plasma with porous hollow fiber having special performance.

[0004]

Patent documents 3 and 4 disclose a filtration method and system using a filter membrane made of regenerated cellulose similarly. All these patent applications propose a method for removing viruses on the basis of size exclusion according to their size. On the other hand, patent document 5 discloses a method for removing viruses under special conditions of a solution using a membrane having pores with a diameter larger than the size of the viruses.

[0005]

Focusing attention on the fact that envelope viruses bond with LDL (low density lipoprotein), patent document 6 proposes

a method for removing the viruses using the filter for removing LDL.

All these methods, however, have drawbacks. Patent documents 5 and 6 described above employ a method of removing viruses by utilizing exclusion or adsorption according to causing the viruses to aggregate under special conditions or causing them to bond with other mixtures. A problem with this method is the limitation to the type of viruses that can be removed. Another problem is an inconstant removing effect according to fluctuation of conditions.

On the other hand, patent documents 1 and 2 disclose methods for separating into viruses and proteins which have a smaller diameter than viruses depending on size thereof, the disclosed membranes is not necessarily satisfied about both virus removal ratio and protein permeating performance. Further, although the methods disclosed in patent documents 3 and 4 are excellent with respect to viruses removal ratio, these methods cannot be applied in practical use due to the limited amount of proteins that can be treated.

[0006]

[Patent document 1] Japanese Patent Application Laid-open No.16837/1986

[Patent document 2] Japanese Patent Application Laid-open No.68176/1988

[Patent document 3] Japanese Patent Application Laid-open No.192368/1989

[Patent document 4] Japanese Patent Application Laid-open No.254205/1989

[Patent document 5] Japanese Patent Application Laid-open

No.28581/1998

[Patent document 6] Japanese Patent Application Laid-open  
No.334037/2000

[0007]

[Problems to be solved by the invention]

An object of the present invention is to provide a safe and industrially effective method for producing a plasma product or a serum product by surely removing viruses from plasma or serum having a potential risk of virus contamination. Another object of the present invention is to provide the plasma product or serum product produced by the method.

[0008]

[Means for solving the problems]

As described above, a method for increasing safety against viruses without denaturing the proteins using a membrane has been studied, however, it has been difficult to provide a method of securing permeability of useful proteins and surely removing serious viruses since the plasma and serum contain a large amount of proteins, many of which are useful proteins with a high molecular weight.

The present inventors have conducted extensive studies to achieve the above objects and have found that viruses can be efficiently removed by introducing a step of removing leucocytes from human plasma or animal plasma used as a raw material before filtering the plasma through a virus removal filter. As a result of further studies, the inventors have completed the present invention.

[0009]

Specifically, the present invention relates to

followings:

(1) A method for producing a human or animal plasma product or serum product comprising:

(a) a step of reducing leukocytes from the plasma or serum originating from a human or animal as a raw material, and separating plasma from the whole blood and

(b) a step of filtering using a virus removal membrane after the step (a).

(2) The method described in (1) above, wherein the virus removal membrane used in step (b) has an average pore diameter of 100 nm or less.

(3) The method described in (1) or (2) above, wherein the step (a) is a leukocyte-reducing step using a leukocyte removal filter.

(4) The method described in any one of (1) to (3) above, wherein the steps (a) and (b) are carried out under the condition of a temperature of 25-40°C.

(5) The method described in any one of (1) to (4) above, wherein the steps (a) and (b) are carried out under condition of a pressure of 98 kPa or less.

(6) The method described in any one of (1) to (5) above, wherein the amounts of blood passing through in the steps (a) and (b) is 100-500 ml.

(7) The method described in any one of (1) to (6) above, wherein the treatment time for the step (b) is 10-40 minutes.

(8) The method described in any one of (1) to (7) above, wherein the virus removal membrane used in the step (b) has an average pore diameter of 75 nm or less.

(9) The method described in any one of (1) to (8) above,

wherein the virus removal membrane used in the step (b) is a combination of a virus removal membrane having an average pore diameter of 75 nm and another virus removal membrane having an average pore diameter of 35 nm subsequent to the former membrane.

(10) A human or animal plasma product or serum product produced by a method comprising the following steps (a) and (b):

(a) a step of reducing leukocytes, and

(b) a step of filtering using a virus removal membrane after the step (a).

[0010]

[Embodiments of the invention]

The present invention is based on the finding that viruses can be surely and efficiently removed, while ensuring permeability of useful components, by reducing leukocytes in advance in case of filtrating plasma before freezing to fresh frozen plasma using a virus removal filter.

The human or animal plasma or serum used as a raw material for producing the plasma product or serum product of the present invention is preferably a fresh raw plasma before freezing. Because plasma stored for a long time before freezing causes a reduction in permeability and in the permeated amount during filtration operation due to proteins, lipids, and the like that have bonded during storage, plasma with a short storage time before freezing is preferable.

[0010]

Any method that can reduce leukocytes may be used in the leukocyte reducing step of the present invention. Among the methods, for example, a method of using ultracentrifugation can

be given, but, a method of using a leukocyte removal filter is preferable due to its simplicity. There are no specific limitations to the materials and the like of the leukocyte removal filter in as much as the filter can reduce leukocytes. For example, polyester nonwoven fabric can be used preferably.

The virus removal filter used in the present invention refers to a membrane having at least a function of separating into viruses and proteins depending on the difference in the size. The virus removal membrane may be made of any material such as regenerated cellulose, polyethylene, polyvinylidene fluoride, and the like, with regenerated cellulose being preferable due to a low protein adsorption.

[0012]

Although there is no unified method and criteria for defining the average pore size of various virus removal membranes used conventionally, the average pore size of the virus removal membrane can be defined in the present invention according to the virus removal performance as follows. Specifically, a virus removal membrane with an average pore size of A nm refers to a membrane that can efficiently remove viruses with a particle diameter of A nm or larger. The above term "efficiently remove" refers to a logarithmic removal ratio ( $LRV = -\log_{10} (\text{virus concentration after filtration} / \text{virus concentration before filtration})$ ) of 3 or more, preferably 4 or more, and more preferably 6 or more. For instance, a virus removal membrane with an average pore size of 100 nm refers to a membrane that can efficiently remove viruses with a particle diameter of 100 nm or larger. Therefore, the virus removal membrane to be used is selected according to the average

particle diameter of the viruses to be removed. For example, specific viruses to be removed by a virus removal filter having an average pore size of 100 nm include the AIDS virus (HIV, average particle diameter: 100-120 nm), pseudorabies virus (PSR, average particle diameter: 120-200 nm), mouse leukemia virus (MuLV, average particle diameter: 120-150 nm), and the like.

[0013]

In order to remove the AIDS virus (HIV) from plasma or serum, a virus removal filter having an average pore size of 100 nm or less is useful. A virus removal membrane with an average pore size of 75 nm or less may preferably reduce the possibility of AIDS virus contamination and further remove even viruses with a particle diameter of 75-100 nm, thereby increasing the safety of the resulting product. Protein permeability is so preferable that it is high as much as possible. The total protein permeability is preferably 70% or more, and more preferably 80% or more.

The virus removal membrane can also remove pathogenic factors and unnecessary impurities in plasma, if an appropriate pore size is selected.

[0014]

The present invention provides a method for producing highly safe plasma from fresh plasma, comprising (a) a step of reducing leukocytes by using a leukocyte removal membrane and the like and (b) a step of removing viruses by filtering using a virus removal filter after the step (a). Although these steps may be carried out using any apparatus and operation, reproducibility of the operation results can be expected and

the quality of plasma after filtration can preferably be stabilized, if the operation is carried out under the controllable condition having (i) a temperature controlling means and (ii) a pressure-applying means. The temperature controlled by the temperature controlling means (i) may be any temperature at which the proteins are not denatured, with a preferable temperature range being from 25 to 45°C. If the temperature during filtration is less than 25°C, it takes a long time for filtration due to an increase in the plasma viscosity. It is difficult to treat within a practically acceptable time. Therefore, a filter temperature of 25°C or more is preferable. If the treatment temperature is higher than 45°C, on the other hand, quality of proteins may unpreferably deteriorate due to heat. A more preferable temperature range is 30 to 37°C.

Although any pressure less than withstand pressure of the membrane may be applied during the treatment, pressure of 98 kps or less is preferable to minimize protein denaturing, with a more preferable pressure being 80 kps or less.

[0015]

Although there are no specific limitations to the amount of plasma treated at one treatment, 100 to 500 ml is usually preferable, because the amount of plasma collectible at one treatment from a single donor is usually 100 to 500 ml. A treatment amount of less than 100 ml at one treatment is unpreferably not economical, whereas an amount of more than 500 ml imposes too great burden on the donor for collecting the plasma from the individual. A more preferable amount is 200 to 400 ml.

The treating time is determined depending on the amount

of treated plasma, the membrane area, and the like and is preferably set 40 minutes or less. When the blood is filtered simultaneously with collection, the treating time of 40 minutes or less imposes only a little burden on the donor as an individual. If the filtration time is too long, it may cause denaturing of the resulting product, in some situations.

When plasma is filtered using a virus removal filter with a pore size of 75 nm and a subsequent virus removal filter with a pore size of 35 nm in the operation described above, dangerous viruses such as the hepatitis C virus (HCV, average particle diameter: 30-60 nm) and the like are removed, and a plasma product which has higher safety can be provided.

Serum obtained by previously removing fibrinogen from plasma is also used as a raw material. A highly safe serum product can also be produced by removing fibrinogen in any step in the plasma product production.

[0016]

[Effects of the invention]

Since the safety against viruses of the plasma product or serum product obtained by the present invention is increased in accordance with the average pore diameter the virus removal membrane used for the filtration, the plasma product or serum product can be used for transfusion as is or can be preserved as frozen fresh plasma, and can be further used for transfusion or as a raw plasma for producing fractional (cut off) products.

[0017]

[Examples]

The present invention will be described by examples.

[Example 1]

Immediately after separating plasma from a donor's blood using a plasma separation collection apparatus (AF104 manufactured by Fresenius), the separated plasma was filtrated through a leukocyte removal filter ("Sepacell" manufactured by Asahi Kasei Corporation), followed by a virus removal filter ("Planova" manufactured by Asahi Kasei Corporation). The "Planova" used had a membrane area of 0.06 sqm. The filtration is carried out first using an average pore size of 75 nm, subsequently using an average pore size of 35 nm. The filtration was performed at a constant flow rate using an OT-601 pump manufactured by JMS Co., Ltd. The filtration temperature was controlled at  $35^{\circ}\text{C} \pm 2^{\circ}\text{C}$ .

250 ml of plasma was treated in 30 minutes under a pressure of 0.3 to 0.6 kg/sqcm. The amount of permeated protein is shown in Table 1. It can be seen from Table 1 that the amount of the permeated protein was 75% or more after filtration using a filter with an average pore size of 75 nm, and that the amount is 90% or more except for F-VIII and 50% or more even for F-VIII after filtration with an average pore size of 35 nm, whereby indicating no problems in practical use.

[0018]

[Comparative Example 1]

An experiment was carried out in the same manner as in Example 1 except for omitting "Sepacell" filtration and "Planova" filtration with an average pore size of 75 nm. Filtration was started using a Planova with an average pore size of 35 nm and a membrane area of 0.06 sqcm. When 50 ml of the sample was filtered thereafter, the pressure increased to more than 1.0 kg/sqcm and the operation was thus discontinued.

[0019]

[Table 1]

Protein	Leukocyte removal filter	Virus removal filter	
		75 nm	35 nm
F-VIII	88%	75%	55%
Globulin	93%	90%	82%
Albumin	100%	100%	100%

[Document Name] Abstract

[Abstract]

[Problems] The present invention relates to a plasma product or a serum product with an extremely low risk of viral contamination and a method for producing the same.

[Means for solving] Before treating plasma or serum to be used as a raw material for producing a plasma product or a serum product using a virus removal filter, leucocytes contaminating the blood are removed. Thus, a plasma product or a serum product with an extremely low risk of viral contamination can be efficiently produced while preventing clogging.

Since clogging scarcely arises, it is possible to carry out efficient filtration without applying an elevated pressure as the filtration proceeds.

[Drawing] None